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Effects of solid-state fermentation using *Aspergillus niger* on yield, total phenolic content, and antioxidant activity of defatted rice bran extract

Muhammad Yusuf Abduh [a,b,*], Sandra Alyssa [a], Ruth Aurelia Butar [a], Irma Septina Sitorus Pane [a], Lili Melani [a], Noor Illi Mohamad Puad [c]

[a] School of Life Sciences and Technology, Institut Teknologi Bandung, Jalan Ganesha 10, Bandung 40132, Indonesia
[b] University Center of Excellence for Nutraceuticals, Bioscience and Biotechnology Research Center, Institut Teknologi Bandung, Jalan Ganesha 10, Bandung 40132, Indonesia
[c] Bioprocess and Molecular Engineering Research Unit, Department of Chemical Engineering and Sustainability, Kulliyyah of Engineering, International Islamic University Malaysia, 50728 Kuala Lumpur, Malaysia

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ABSTRACT

This study aimed to determine the effects of defatted rice bran fermentation using *A. niger* towards extract yield and bioactivity against various solvents. Solid-state fermentation of defatted rice bran using *A. niger* was carried out in dark conditions at 27 °C for 7 d. The results showed that the fermentation process reduced the hemicellulose, cellulose, and lignin content by 22.36 %, 42.47 %, and 22.96 % after 7 d of fermentation. Extraction of phenolic compounds from the fermented defatted rice bran was carried out by a maceration at 40 °C for 180 min using methanol (80 %), ethanol (80 %), and acetone (40 %). The fermentation increased the yield of defatted rice bran extract by up to 81 % (when using ethanol (80 %) as the extracting solvent) as compared to without fermentation. The total phenolic content and ferulic acid of the defatted rice bran extract also increased upon fermentation up to 339 % and 58 times when the extraction was carried out using ethanol (80 %). The antioxidant activity of the defatted rice bran extracts also increased upon fermentation with the lowest value of IC_{50} (102.19 ppm) obtained when the extraction was carried out using ethanol (80 %).

1. Introduction

Rice (*Oryza sativa*) is one of the staple food crops for most of the world's population. The first grain of rice goes through a milling process to produce 70 % rice, 20 % husk, and the rest is rice bran (8–10 %) which is a cream or light brown layer between the rice husk and rice grain Chen et al. (2012). Rice bran contains 12–23 % lipid (Spaggiari et al., 2021) which can be extracted to produce rice bran oil as the main product and defatted rice bran as the byproduct. The defatted rice bran contains carbohydrates, protein, lignin and phytic acid. The defatted rice bran is also rich in phenolic compounds such as γ-oryzanol, ferulic acid, caffeic acid, tricin and coumaric acid (Henderson et al., 2012) that can be extracted and valorized in various applications.

Currently, there are several studies that report the effects of different solvent and fungal fermentation on extraction yield and bioactivity of rice bran extract. However, no studies have reported the effects of fermentation on the degradation of lignocellulosic components of defatted rice bran and its correlation with the phenolic extract yield and composition of the extract particularly ferulic acid. The effects of fermentation on the concentration of ferulic acid is interesting to be investigated in this study because it has many pharmacological effects such as antioxidant activity, anti-inflammatory, vasodilating, antithrombotic, anti-microbial, anti-allergic, anti-viral, hepatoprotective and anti-cancer (Gupta et al., 2021).

Devi and Arumughan (2007) have investigated the effects of different solvents on the yield, total phenols, oryzanols and ferulic acid from defatted rice bran. The study found that methanol was the most effective solvent to obtain 0.233 mg g^{-1} of ferulic acid after 10 h of Soxhlet extraction. In another study, Chatta et al. (2006) have investigated the effects of extracting solvents particularly methanol (80 %) and acetone with different concentration toward the extraction yield of active compounds from rice bran. The study discovered that the yield of rice bran extract varies from 11.21 to 16.71 % and recommends methanol (80 %) as the most suitable solvent for extraction of antioxidant compounds from rice bran. Iqbal et al. (2005) also used methanol (80 %) to extract antioxidant compounds from different rice bran varieties and

* Corresponding author at: School fo Life Sciences and Technology, Institut Teknologi Bandung, Jalan Ganesha 10, 40132 Bandung, Indonesia.
E-mail address: yusuf_abduh@itb.ac.id (M.Y. Abduh).

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discovered that total phenolic content lies in the range of 2.51–3.59 mg g^{-1} of bran extracts.

In another study, Zhao et al. (2018) demonstrated that defatted rice bran contains bound and free phenolic content of phenolic content of 2.64 and 5.06 mg g^{-1}. The bound phenolic can be released from the macromolecules and plant cell walls of defatted rice bran by microbial fermentation (Ritthibut et al., 2021). Currently, no studies have reported on the effects of microbial fermentation towards the phenolic content of defatted rice bran. Potential microbes that can be used to pre-treat the defatted rice bran prior to the extraction process are *Aspergillus* strains that have been widely used for improvement of total phenolic content and antioxidant activity. Ritthibut et al. (2021) have investigated the effects of solid-state fermentation of rice bran for 14 d using *A. brasiliensis, A. awamori*, and *A. sojae* followed by extraction with ethanol (95 %). The study observed that the fermentation increased the total phenolic content and ferulic acid of the rice bran extract up to 3 times and 63 times higher than the unfermented rice bran.

In another study, Abduh et al. (2020) have investigated the ability of *A. niger, A. Awamori* and *A. oryzae* to grow and release volatile compounds from lemon peels under solid-state fermentation conditions. The study reported that *A. niger* can degrade lignin up to 5.4 % dry weight, higher than the *A. Awamori*, and *A. oryzae*. A study by Huynh et al. (2016) reported that fermentation of cauliflower outer leaves by *A. sojae* augmented the total phenolic content and changed the phenolic profile. Gulsunoglu et al. (2020) also found that fermentation of apple peel using *A. tubingensis, A. niger, A. japonicus* and *A. aculeatus* for 7 d increased phenolic content and antioxidant activity of the apple peel extract. The study also highlighted that fermentation of apple peel with *A. niger* yield the highest phenolic content and flavonoid content.

The findings indicate that fermentation with *Aspergillus* spp. can be considered as a good pre-treatment step because it can produce cellulase, xylanase and ligninase enzymes that can degrade lignocellulosic components in the plant cell walls and consequently the phenolic compounds can be more easily extracted (Berquist et al., 2002; Najafpour et al., 2007). In this study, *A. niger* was particularly chosen based on the results of previous studies that suggest *A. niger* can degrade lignin and increased the phenolic content better than the other *Aspergillus* species. This study also focused particularly on ferulic acid because ferulic acid is the major phenolic acid present in the defatted rice bran (Ritthibut et al., 2021). Hence, this study aimed to determine the effects of fermentation of defatted rice bran using *A. niger* on the lignocellulose component of the defatted rice bran and extract yield as well as total phenolic content, antioxidant activity and ferulic acid content of the defatted rice bran extract.

2. Materials and methods

2.1. Materials

Rice (*Oryza sativa*) bran used in this study was obtained from a local rice mill located in Bandung district, West Java. Indonesia whereas *A. niger* was obtained from the School of Life Sciences and Technology, Institut Teknologi Bandung, Indonesia. Calcium chloride dihydrate ($CaCl_2{\cdot}2H_2O$), potassium phosphate ($KHPO_4$), glucose, $MgSO_4{\cdot}7H_2O$, sodium chloride (NaCl), distilled water, gallic acid, sodium carbonate (Na_2CO_3), cupric sulfate ($CuSO_4$), sulfuric acid (H_2SO_4), boric acid (H_3BO_3), potassium sulfate (K_2SO_4), sodium hydroxide (NaOH), methanol, ethanol, acetone, Folin-Ciocalteu reagent, and n-hexane were obtained from Merck (Germany) while DPPH reagent (2,2-diphenyl-1-picrylhydrazyl) was from Sigma-Aldrich Chemicals (USA).

2.2. Preparation of defatted rice bran

The rice bran obtained in this study was separated from the rice husks and then dried at 60 °C for 4 h. After that, the dried rice bran was crushed using a blender and then sieved using mesh 60 to obtain a homogeneous size of rice bran (~ 0.25 mm). After that, the homogeneous rice bran was subjected to Soxhlet extraction using n-hexane for 6 h to remove the oil content in the rice bran (Abduh et al., 2016) followed by evaporation of the n-hexane in a fume hood for 24 h.

2.3. Proximate analysis of defatted rice bran

Proximate analysis was carried out to determine the ash, crude protein, fat and carbohydrate content of the defatted rice bran. The ash content was determined by burning the samples in a furnace at 600 °C for 6 h (AOAC (2005) method 942.06). The protein content of the samples was obtained using the Kjeldahl method 960.52 (AOAC, 2005). whereas the fat content was determined using the Soxhlet method 948.22 (AOAC, 2005). The carbohydrate content was calculated using the by-difference method.

2.4. Solid-state fermentation of defatted rice bran with A. niger

Solid-sate fermentation of the defatted rice bran was carried out in a fermenter tray containing 100 g of defatted rice bran and 15 g of *A. niger* culture with a concentration of 3.2×10^8 CFU g^{-1} mixed with a solution consisting of 3.4 g yeast extract, 0.09 g $CaCl_2$.$2H_2O$, 3.3 g $MgSO_4{\cdot}7H_2O$, 1.3 g KH_2PO_3, 16.7 g glucose and 175 mL of distilled water. Since *A. niger* typically reached a stationary phase after 5 d of fermentation, the fermentation process in this study was carried out for 7 d as suggested by Gulsunoglu et al. (2020). Samples were taken periodically (day 0,1, 3, 5, 7) followed by washing with water and dried at 60 °C in an oven until the moisture content of the samples were around 10 % as determined using a weight loss method prior to further analysis (Abduh et al., 2024).

2.5. Determination of total moisture content

The total moisture content of samples was determined gravimetrically using a weight loss method. Samples were weighed before drying in an oven at 105 °C overnight until constant weight was obtained. The moisture content of the samples was determined by calculating the difference between wet and dry weight of the samples divided by the wet weight of the samples as shown in Eq. (1).

$$\textit{Moisture content}\ (\%) = \frac{m_{wet}(g) - m_{dry}(g)}{m_{wet}(g)} \times 100\% \quad (1)$$

Where m is mass in gram (g).

2.6. Determination of lignocellulosic composition of defatted rice bran

The lignocellulosic composition in the samples was determined using the procedures as suggested by Abduh et al. (2021). A flask containing 1 g dry sample (m_1) was mixed with 150 mL of distilled water and then heated at 100 °C under reflux for 2 h. The mixture was then filtered using Whatman filter paper and the residue was dried in an oven at 105 °C, and the dry weight was recorded (m_2). Then, the dried residue was mixed with 150 mL of 0.5 M H_2SO_4 and heated at 100 °C under reflux for 2 h. The mixture was filtered using a Whatman filter paper, and then the residue was dried in an oven at 10 5 °C and the weight was recorded (m_3). The samples were then immersed in 10 mL of 72 % (volume basis) H_2SO_4 for 4 h at room temperature (27 °C). After 4 h, the mixture was diluted to 0.5 M H_2SO_4 and heated again under reflux at 100 °C for 2 h. The mixture was filtered using a Whatman filter paper and the residue on the filter paper was dried in an oven at 105 °C, and the dry weight was recorded (m_4). The dried residue was reduced to ash using a furnace at 575 °C until a constant mass was obtained (m_5). Hemicellulose, cellulose, and lignin content in the samples were calculated using Eqs. (2) to (4).

$$Hemicellulose\ (\%) = \frac{m_2(g) - m_3(g)}{m_1(g)} \times 100\% \quad (2)$$

$$Cellulose\ (\%) = \frac{m_3(g) - m_4(g)}{m_1(g)} \times 100\% \quad (3)$$

$$Lignin\ (\%) = \frac{m_4(g) - m_5(g)}{m_1(g)} \times 100\% \quad (4)$$

2.7. Extraction of phenolic compounds

Extraction of phenolic compounds from defatted rice bran was carried out using an incubator shaker and different solvents particularly methanol (80 %), ethanol (80 %) and acetone (40 %) at 40 °C with a sample to solvent ratio of 1:10 (weight: volume) for 3 h. After that, the mixture was filtered using a Whatman filter paper followed by evaporation of the solvent using a rotary evaporator to obtain a concentrated phenolic extract (Schmidt et al., 2014). The yield of the defatted rice bran extract was calculated using Eq. (5) where m_1 is the mass of the rice bran sample and m_2 is the mass of the concentrated phenolic extract.

$$\boldsymbol{Yield}\ (\%) = \frac{\boldsymbol{m_2(g)}}{\boldsymbol{m_1(g)}}\boldsymbol{x}100\% \quad (5)$$

2.8. Determination of total phenolic content

Total phenolic content of the defatted rice bran extract was determined using the Folin-Ciocalteu method. Initially, a standard curve was prepared by dissolving 0.1 g of gallic acid in 100 mL of solvent to obtain a standard solution of gallic acid and then diluted using a serial dilution to obtain gallic acid concentrations of 5, 20, 40, 60, 80, and 100 ppm. After that, 1 mL of the extract solution was mixed thoroughly with 5 mL of 10 % Folin-Ciocalteu solution and 4 mL of 7.5 % sodium carbonate and incubated for 30 min in the dark. The total phenolic content in the sample was measured using a UV–VIS spectrophotometer at a wavelength of 765 nm and expressed as mg of gallic acid equivalent (GAE) per g of sample as calculated using Eq. (6) (Agbor et al., 2014; Abduh et al., 2023).

$$Total\ phenolic\ content\left(\frac{mg\ GAE}{g\ sample}\right) = \frac{V(mL)}{m\ (g)} \times TPC\left(\frac{mg}{mL}\right) \times yield(\%) \quad (6)$$

Where m is the mass of the sample (g), V is the volume of the sample (mL), and the yield is the extraction yield for each sample (%).

2.9. Determination of antioxidant activity

Antioxidant activity of the defatted rice bran extract was determined based on the scavenging ability of 2,2-diphenylpicrylhydrazyl (DPPH) free radicals and expressed as the concentration of IC_{50} (Abduh et al., 2023). Initially, the sample was diluted in the solvent to obtain solutions with concentrations of 50, 100, 200, 300, and 400 ppm. After that, 1 mL of each solution concentration was mixed thoroughly with 4 mL of 0.05 mM DPPH reagent. The sample was then incubated for 30 min in a dark room. The absorbance of the solution was measured using a UV–Vis spectrophotometer at a wavelength of 517 nm. The antioxidant activity of the sample is expressed as DPPH scavenging activity as calculated by Eq. (7). The value of IC_{50} can be calculated from the linear curve of DPPH free radical scavenging activity measured against the concentration of the samples.

$$Antioxidant\ activity = DPPH\ Scavenging\ activity\ (\%) = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100\% \quad (7)$$

2.10. Determination of ferulic acid content

Ferulic acid content in the defatted rice bran extract was determined using the High-Performance Liquid Chromatography (HPLC) with a C18 reversed phase column (150 × 4.6 mm × 5 µm). Approximately 0.02 g of the extract was dissolved in 10 mL of methanol (HPLC grade) using a 150 W ultrasonic water bath for 3 h. Ferulic acid standard solutions (0.3125–5 ppm) were prepared by diluting the ferulic acid standard in the methanol. The mobile phase was a mixture of 30 % methanol and 70 % deionized water containing 1 % acetic acid. Prior to injection, the sample was filtered using a 0.45 µm polytetrafluoroethylene hydrophilic syringe filter. After that, approximately 10 µL of the filtrate was injected into the HPLC machine. Ferulic acid content was measured using a 320 nm wavelength with a mobile phase flow rate of 1 mL/min while the column temperature was maintained at 40 °C throughout analysis. The analysis was carried out for 35 min with the retention time of ferulic acid was 28 min. The results of the standard measurements were plotted into a linear regression where y is the peak area and x is the concentration of ferulic acid. The linear regression equation was then used to determine the concentration of ferulic acid in the sample (Li, 2014).

2.11. Determination of polarity index

The polarity index of methanol (80 %), ethanol (80 %) and acetone (40 %) used in this study was calculated using Eq. (8) as suggested by Musa et al. (2009).

$$P_m = \emptyset_1 P_1 + \emptyset_2 P_2 \quad (8)$$

Where P_m is the polarity index of the solvent mixture, $\emptyset_1$ and $\emptyset_2$ are the volume fractions of solvent 1 (methanol, ethanol, acetone) and solvent 2 (water) whereas P_1 and P_2 are the polarity indices of solvent 1 (methanol, ethanol, acetone) and solvent 2 (water). According to Musa et al. (2009), the polarity index for pure acetone, methanol, ethanol, and water are 5.1, 5.1, 5.2 and 10, respectively.

2.12. Statistical analysis

Statistical analysis was carried out in this study using SPSS Statistics 26 software. A One-way ANOVA was carried out to determine the significance of the results ($P < 0.05$) obtained from the extraction of phenolic compounds from the defatted rice bran using different solvents against fermentation time. Each fermentation and assay had three replications. A Duncan's test was also carried out to determine whether there are significant differences between data from one group to another while Pearson's Coefficient Correlation and *P*-values were identified to determine the correlation of the research parameters.

3. Results and discussions

3.1. Proximate and lignocellulose composition of defatted rice bran

The defatted rice bran contains 11.88 ± 0.67 % moisture, 53.38 ± 4.89 % carbohydrates and traces of crude fat that are within range of values reported in previous studies (Ly et al., 2023; Sairam et al., 2011; Nugrahani et al., 2019, see supplementary materials, Table S1). However, the defatted rice bran contains a considerable amount of ash (14.87 ± 1.2 %) and protein (19.87 ± 3.29 %) that are slightly higher than the range of 10.1–11.5 % for ash and 13.6–18.31 % for protein as reported in previous studies (Zhao et al., 2018; Ly et al., 2023). The defatted rice bran also contains 34.13 ± 2.12 % hemicellulose, 19.60 ± 1.23 % cellulose and 14.60 ± 2.26 % lignin. The cellulose and lignin content of the defatted rice bran are within range of 15.5 – 34.0 % and 11.5–24.8 % as reported in previous studies whereas the hemicellulose content is slightly higher than the range of 21.8–31.1 % reported in the literature (Sunphorka et al., 2012; Gupte et al., 1997; Ghodrat et al., 2017, see

supplementary materials, Table S2). Slight differences in the values for proximate and lignocellulose composition may be due to the different varieties of rice as well as different cultivation conditions (Sunphorka et al., 2012; Gupte et al., 1997; Ghodrat et al., 2017).

3.2. Effects of fermentation time on the degradation of lignocellulose

Fig. 1 shows the effects of fermentation with *A. niger* on the composition of hemicellulose, cellulose, and lignin in the defatted rice bran. In general, the lignocellulose content of the fermented defatted rice bran exhibits a decreasing profile throughout the fermentation process. The hemicellulose content decreased from 34.13 ± 2.12 % to 26.5 ± 1.10 % whereas the cellulose and lignin decreased from 19.60 ± 1.23 % and 14.60 ± 2.26 % to 15.10 ± 2.23 % and 8.40 ± 2.75 %, respectively. After 7 d of fermentation, lignin experienced the highest percentage of decrease (42.47 %) followed by cellulose (22.96 %) and hemicellulose (22.36 %). As such demonstrates that the fermentation process has a significant effect ($P < 0.05$) in degrading lignocellulose of the defatted rice bran. Previous studies have shown *A. niger* produces lignocellulosic degrading enzymes such as laccase enzyme that can degrade lignin and endoglucanase enzyme can break the β1,4-glycosidic bond in the amorphous region of cellulose to release the reducing and non-reducing ends of the chain (Dashtban et al., 2009; Faraco, 2013; Iqbal et al., 2018). In addition, *A. niger* also has been reported to secrete hemicellulose degrading enzymes such as Exo-β−1,4-mannosidase, feruloyl esterase, and α-L-arabinofuranosidase as well as xylanase which can degrade xylan, the main component of hemicellulose in plant cell walls into xylose (Beg et al., 2001; Sanchez et al., 2009).

3.3. Effects of fermentation time and solvent on the yield of defatted rice bran extract

Fig. 2 shows the effects of different extracting solvent and fermentation time with *A. niger* on the yield of defatted rice bran extract. In general, the fermentation enhanced the yield of defatted rice bran extract as compared to the non-fermented samples due to the degradation of the lignocellulose (Fig. 1). From Fig. 2, it can be observed that the highest yield for unfermented samples was obtained when the extraction was carried out using methanol as a solvent (8.93 ± 0.335 %) followed by acetone (7.76 ± 1.52 %) and ethanol (6.40 ± 0.36 %). As such may be influenced by the polarity of phenolic compounds present in the

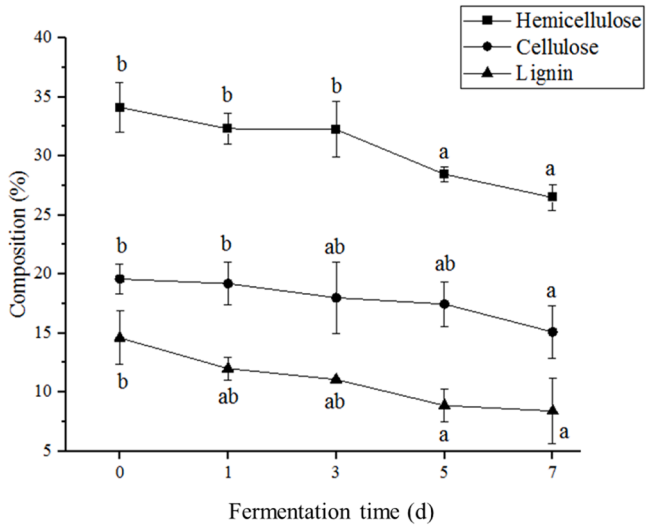

Fig. 1. Hemicellulose, cellulose, and lignin content of defatted rice bran during fermentation with *A. Niger*.
Note: letter notation (a and b) indicates significant difference ($P < 0.05$) in the same compound.

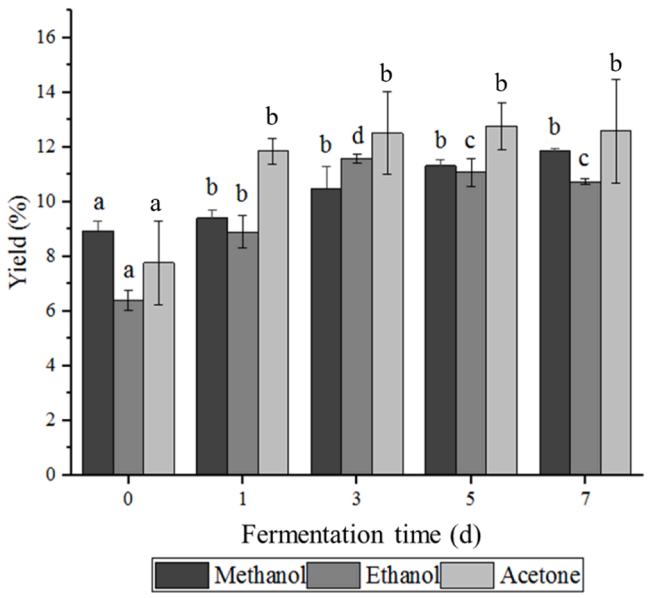

Fig. 2. Yield of defatted rice bran extract for different extracting solvents and fermentation time with *A. niger*.
Note: letter notations (a, b, c and d) indicate a significant difference ($P < 0.05$) in the same solvent.

defatted rice bran that are more attracted to methanol as compared to ethanol and acetone (Farooq et al., 2022). Currently, no studies have reported the effects of fermentation time and solvent on the yield of defatted rice bran extract. However, Chatha et al. (2006) had previously investigated the effects of non-defatted rice bran extraction using methanol and acetone and reported that the rice bran extract yield varies from 10.02 to 16.71 %, with methanol providing a higher yield compared to acetone. A higher yield reported by Chatta et al. (2006) may be because the extraction was carried out for 48 h whereas in this study, the extraction was only carried out for 3 h.

However, upon fermentation, the yield of defatted rice bran extract investigated in study increased up to 81 % as compared to the unfermented samples for extraction with ethanol (80 %), whereas extraction using acetone (40 %) and methanol (80 %) increased the yield up to 64 % and 33 %. Statistically, the fermentation has a significant influence ($P < 0.05$) on increasing the yield reaching a maximum value of 12.76 ± 0.84 % for acetone on day 5, 11.87 ± 0.07 % for methanol (80 %) on day 7 and 11.59 ± 0.16 % for ethanol (80 %) on day 3. The results highlighted that pre-treatment of defatted rice bran using *A. niger* greatly increased the defatted rice bran yield particularly when using acetone (40 %) as compared to methanol (80 %) and ethanol (80 %). According to Musa et al. (2009), the presence of water in the extraction solvent can enhance the extraction efficiency of antioxidant compounds. Musa et al. (2009) observed that acetone (50 %) gave the highest extraction efficiency of antioxidant compounds from pink-flesh guava followed by acetone (70 %) and pure acetone (100 %) due to higher polarity index. In this study, the polarity index of acetone (40 %) is 8.04, higher than the polarity index of 6.16 for ethanol (80 %) and 6.08 for methanol 80 % which results in a higher yield when using acetone (40 %). Similarly, Chatta et al. (2006) also obtained a higher rice bran extract yield of 11.21 ± 0.50 % when using acetone (80 %) as compared to 10.02 ± 0.82 % when using pure acetone (100 %).

According to Ritthibut et al. (2021) fermentation using *Aspergillus* spp. changed the composition and concentration of bioactive compounds in the rice bran extract. During the fermentation, different enzymes with lytic activity induced the release of various bioactive compounds such as kojic acid and oryzanol. Possibly, the released compounds upon fermentation have a greater affinity towards acetone (40 %) that has a higher polarity index which results in a higher yield as

compared to methanol (80 %) and ethanol (80 %). Nevertheless, further studies are needed to understand the mechanisms of how fermentation can greatly influence the effects of solvent on the yield of defatted rice bran extract.

3.4. Effects of fermentation time and solvent on total phenolic content and antioxidant activity of defatted rice bran extract

Fig. 3 shows the effects of different extracting solvent and fermentation time with *A. niger* on the total phenolic content of defatted rice bran extract. From the figure, it can be observed that the highest total phenolic content for unfermented samples was obtained when the extraction was carried out using methanol (80 %) as a solvent (3.67 ± 0.45 mg GAE g^{-1}) followed by acetone (40 %) (3.64 ± 0.13 mg GAE g^{-1}) and ethanol (80 %) (2.49 ± 0.17 mg GAE g^{-1}). These values are slightly lower than the total phenolic content of defatted rice bran (7.69 ± 0.17 mg GAE g^{-1}) reported by Zhao et al. (2018). Hower upon fermentation, the total phenolic content of defatted rice bran investigated in study increased up to 339 % as compared to unfermented samples for extraction with ethanol, whereas extraction using methanol and acetone increased the total phenolic content up to 69 % and 27 %.

The results highlighted that pre-treatment of defatted rice bran using *A. niger* greatly increased the total phenolic content of defatted rice bran yield particularly when using ethanol (80 %) as compared to methanol (80 %) and acetone (40 %). As discussed in the previous section, ethanol (80 %) has a slightly higher polarity index than methanol (80 %). As such may contribute to the higher total phenolic content of defatted rice bran extract when the extraction was carried out using ethanol (80 %) as compared to methanol (80 %). As for acetone (40 %), the lower total phenolic content despite having the highest polarity index might indicate the presence of compounds other than phenol such as oryzanols (Devi & Arumughan, 2007). Nevertheless, further studies are needed to understand the mechanisms of how fermentation can greatly influence the effects of solvent on the total phenolic content of defatted rice bran extract.

Statistically, the fermentation has a significant influence ($P < 0.05$) on increasing the total phenolic content reaching a maximum value of 6.18 ± 0.35 mg GAE g^{-1} for methanol on day 7, 10.93 ± 0.31 mg GAE g^{-1} for ethanol on day 3 and 4.62 ± 0.12 mg GAE g^{-1} for acetone on day 5.

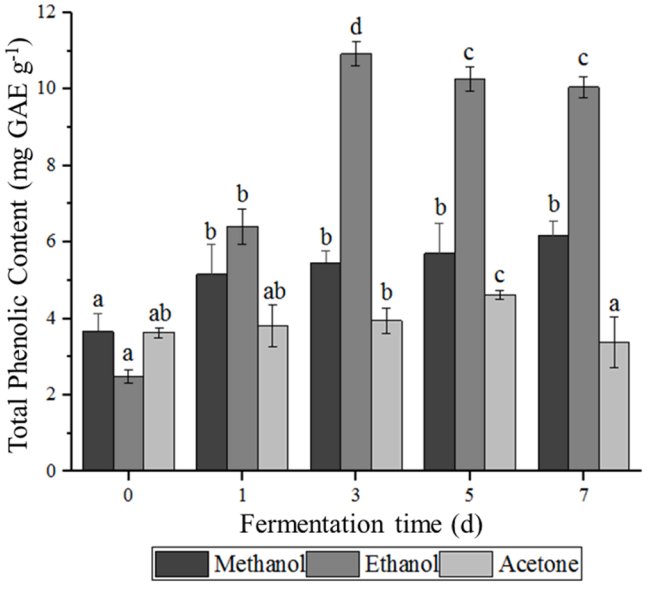

Fig. 3. Total phenolic content of defatted rice bran extract for different extracting solvents and fermentation time with *A. niger*.
Note: letter notations (a, b, c and d) indicate significant difference ($P < 0.05$) in the same solvent.

The results resemble the previous findings by Iqbal et al. (2005) that fermentation of rice bran with *A. sojae* for 14 d increased the total phenolic content of the rice bran extract using ethanol (95 %) up to 3 times higher than the unfermented rice bran. According to Iqbal et al. (2005), fermentation of rice bran with fungus induced the release of free phenolic compounds from bound polymetric matrices which result in an increase of total phenolic content in the rice bran extract. In another study, Yin et al. (2017) demonstrated that the use of *A. oryzae* and *A. niger* culture increased phenolic content of fermented wheat bran up to 12–15 times higher as compared to the unfermented samples. From that study, Yin et al. (2017) also observed that *A. niger* can release phenolic acids bound to the wheat bran better than the *A. oryzae* after 7 d of fermentation.

The percentage of radical scavenging activity of the unfermented and fermented defatted rice bran extract were measured using a DPPH method to determine the antioxidant activity of the samples expressed in terms of IC_{50} (ppm) and the results are shown in Fig. 4. From the figure, it can be noted that the antioxidant activities of the defatted rice bran extract increased upon fermentation as reflected by the lower values of IC_{50} for all fermented samples than the unfermented sample which indicates high antioxidant activity. The lower the absorbance in the DPPH solution and the higher the percentage of antioxidant activity, the lower is the value of the IC_{50}, the concentration of the extract required to inhibit 50 % oxidation by free radicals.

The antioxidant activity of the defatted rice bran extract shows a similar profile with the total phenolic content shown in Fig. 3. The highest antioxidant activity for unfermented samples was obtained when the extraction was carried out using methanol (80 %) as a solvent (240.23 ± 2.12 ppm) followed by acetone (40 %) (342 ± 61.84 ppm) and ethanol (80 %) (384.50 ± 69.49 ppm). A similar profile indicates that the phenolic compounds were responsible for the antioxidant activity of the rice bran extract as also observed by Iqbal et al. (2005). Upon fermentation, the radical scavenging activity increased as reflected by the decreasing values of IC_{50} up to 73 % as compared to unfermented samples for extraction with ethanol (80 %), whereas extraction using methanol (80 %) and acetone (40 %) decreased the

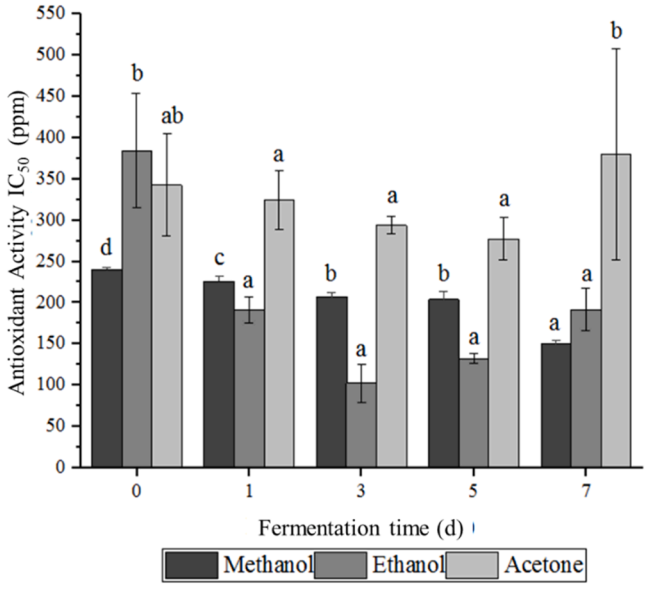

Fig. 4. Antioxidant activity (IC_{50}) of defatted rice bran extract for different extracting solvents and fermentation time with *A. niger*. An IC_{50} is the concentration required to inhibit 50 % of biological activity. A IC_{50} indicates stronger activity because the biological activity can be inhibited at a lower concentration.
Note: letter notations (a, b, c and d) indicate significant difference ($P < 0.05$) in the same solvent.

values of IC_{50} up to 37 % and 23 %, respectively. These results agree with the previous study by Ritthibut et al. (2021) that the antioxidant activities of rice bran extract increased upon fermentation as microorganisms degrade the complex structure of phenolic compounds and the released free compounds can act as antioxidants.

Statistically, the fermentation has a significant influence ($P < 0.05$) on decreasing the values of IC_{50} reaching a minimum value of 150.24 ± 3.82 ppm for methanol on day 7, 102.19 ± 22.74 ppm for ethanol on day 3 and 277.59 ± 25.54 ppm for acetone on day 5. These results are in line with the previous findings by Iqbal et al. (2005) that fermentation of rice bran with *A. sojae* for 10 d increased the radical scavenging activity of the rice bran extract up to 4 times higher than the unfermented rice bran. In another study, Ritthibut et al. (2021) reported that fermentation of rice bran using *A. brasiliensis* increased the radical scavenging activity from 15.7 % to 33.8 % after 8 d of fermentation.

Ferulic acid is one of the major phenolic compounds contained in rice bran with strong antioxidant activity and the effects of fermentation using *A. niger* on the ferulic acid content of defatted rice bran is shown in Fig. 5. From the figure, it can be observed that the highest ferulic acid content for unfermented samples was obtained when the extraction was carried out using acetone (40 %) as a solvent (0.11 ± 0.004 mg g^{-1}) followed by methanol (80 %) (0.004 ± 0.0002 mg g^{-1}) and ethanol (80 %) (0.003 ± 0.0008 mg g^{-1}). These values are much lower than the free ferulic acid content in defatted rice bran of 0.03 ± 0.002 mg g^{-1} as reported by Zhao et al. (2018) which may be due to different extraction conditions. However, the values of ferulic content obtained in this study (0.00002 to 0.0002 mg mL^{-1}) are still within the range of 0.0004 to 0.002 mg mL^{-1} as reported by Abd Razak et al. (2017) and Ritthibut et al. (2021) for non-defatted rice bran.

Upon fermentation carried out in this study, the ferulic acid content increased to 58 times as compared to the unfermented samples for extraction with ethanol (80 %), whereas extraction using methanol (80 %) and acetone (40 %) increased the ferulic acid content up to 37 times and 20 times. Statistically, the fermentation has a significant influence ($P < 0.05$) on increasing the ferulic content reaching a maximum value of 0.15 ± 0.008 mg g^{-1} for ethanol, 0.13 ± 0.008 mg g^{-1} for methanol and, 0.11 ± 0.008 mg g^{-1} for acetone on day 3. The results resemble the previous findings by Ritthibut et al. (2021) that fermentation of non-defatted rice bran using *A. brasiliensis* and *A. awamori* increased the ferulic acid content in the early stage of fermentation but then gradually decreased in the later stage. From Fig. 5, it can be noticed that the ferulic acid content in each extract reached a maximum value on day 3 whereas the highest total phenolic content and strongest antioxidant activity of the extracts were obtained on day 3, 5 and 7, depending on the extraction solvent. As such might indicate the presence of phenolic compounds other than ferulic acid such as coumaric acid, sinapic acid, benzoic acid and cinnamic acid (Ritthibut et al., 2021) that contributed to the profile of total phenolic content and antioxidant activity shown in Figs. 3 and 4. This agrees with the results of Pearson's correlation analysis that there is no significant relationship between ferulic acid content in the extract with the total phenolic content, and antioxidant activity of defatted rice bran extract (see supplementary materials, Table S3).

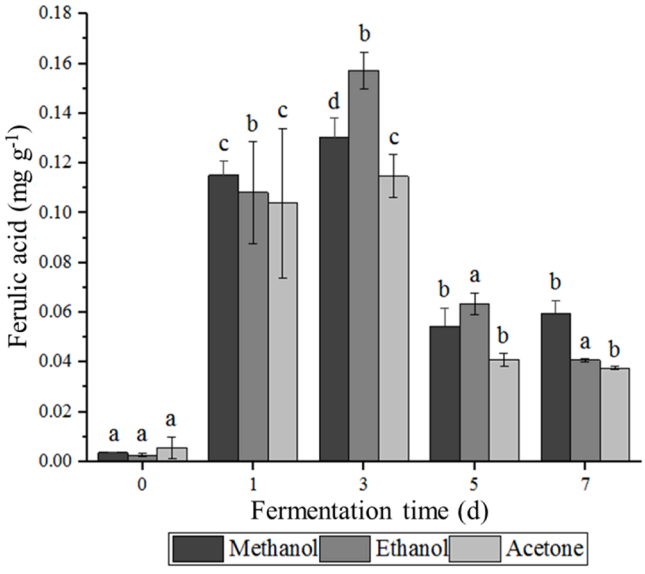

Fig. 5. Ferulic acid content of defatted rice bran extract for different extracting solvents and fermentation time with *A. niger*.
Note: letter notations (a, b, c and d) indicate significant difference ($P < 0.05$) in the same solvent.

Ritthibut et al. (2021) reported that fermentation of rice bran using different *Aspergillus* species results in the different phenolic composition in the rice bran extracts. During fermentation, different fungal species would release different cell wall hydrolytic enzymes and consequently the phenolic composition would be different when fermentation of rice bran was carried out using different species. For instance, fermentation of rice bran using *A. brasiliensis* increased the coumaric acid content from 0.0002 mg mL^{-1} (non-fermented samples) to 0.0044 mg mL^{-1} as after 2 d of fermentation before gradually decreased and not detected after 5 d of fermentation. The study also reported that fermentation of rice bran using *A. awamori* increased the ferulic acid content and reached a maximum value of 0.025 mg mL^{-1} after 4 d of fermentation before gradually decreased, but a fluctuating pattern was observed when the fermentation was carried out using *A. sojae*. According to Ritthibut et al. (2021), the changes in phenolic acid profile depend on fungal species and fermentation time. Prolonged fermentation may result in phenolic compounds being consumed or degraded by the fungus (Ritthibut et al., 2021; Schmidt et al., 2014).

3.5. Correlation analysis for defatted rice bran extract yield, total phenolic content and antioxidant activity

Pearson's Correlation Coefficient (r) was analyzed statistically to evaluate the relationship between defatted rice bran extract yield, total phenolic content, and antioxidant activity and the results are shown in Table 1. From the table, it can be observed that the total phenolic content has a positive correlation with the defatted rice bran extract yield for methanol, ethanol, and acetone solvents which means that the yield positively influences the total phenolic content. The correlation test between total phenolic content and defatted rice bran extract yield gave a P value < 0.05 for methanol and ethanol solvents indicating a significant effect between the yield and total phenolic content. The results highlight that the total phenolic content of the extract increased with increasing defatted rice bran extract yield.

A negative correlation was obtained between the total phenolic content and the IC_{50} value of the antioxidant activity which means that the total phenolic content is inversely proportional to the IC_{50} value of

Table 1
Pearson's correlation coefficient for extract yield, total phenolic content, and antioxidant activity.

Solvent	Parameter	Pearson Correlation Coefficient (r); *P*-value Total Phenolic Content
Methanol 80 %	Yield	0.892; 0.042*
	Antioxidant activity, IC_{50}	−0.830; 0.082
Ethanol 80 %	Yield	0.9978; 0.0001*
	Antioxidant activity, IC_{50}	−0.9284; 0.0227*
Acetone 80 %	Yield	0.3446;0.568
	Antioxidant activity, IC_{50}	−0.916;0.029*

*Significantly correlated ($P < 0.05$).

antioxidant activity. The correlation test between total phenolic content and the IC_{50} of antioxidant activity gave a P value < 0.05 for ethanol and acetone solvent indicating a significant effect between the antioxidant activity and total phenolic content.

4. Conculsion

In brief, fermentation of defatted rice bran using *A.niger* successfully reduced the hemicellulose, cellulose, and lignin content of the defatted rice bran after 7 d of fermentation. The fermentation increased the defatted rice bran extract yield for all solvents. The fermentation also increased the total phenolic content and antioxidant activity of the defatted rice bran extract and reached maximum values on different fermentation time for different extracting solvents. The antioxidant activity of the defatted rice bran extract shows a similar profile with the total phenolic content which indicates that the phenolic compounds were responsible for the antioxidant activity of the defatted rice bran extract. Upon fermentation, the ferulic acid content increased to 58 times on day 3 as compared to unfermented samples. The results suggest that defatted rice bran can be valorized as an alternative source of phenolic compounds with good bioactivity. Bioactive compounds other than ferulic acid were not examined in this study. Potential future study can be directed to investigate the effects of fermentation on the composition various bioactive compounds such as γ-oryzanol and coumaric acid. The results will be very helpful for potential valorization of defatted rice bran as an alternative source of phenolic compounds with good bioactivity.

Declaration

Author contributions statement

a. Muhammad Yusuf Abduh: Conceived and designed the experiments, analyzed and interpreted the data, contributed materials, analysis tools or data, and wrote the paper
b. Sandra Alyssa: Performed the experiments, analyzed and interpreted the data, and wrote the paper.
c. Ruth Aurelia Butar: Performed the experiments, analyzed and interpreted the data, and wrote the paper.
d. Irma Septina Sitorus r: Performed the experiments, analyzed and interpreted the data, and wrote the paper.
e. Lili Melani: Conceived and designed the experiments and wrote the paper
f. Noor Illi Mohamad Puad: Wrote the paper

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Additional information

No additional information is available for this paper.

CRediT authorship contribution statement

Muhammad Yusuf Abduh: Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Sandra Alyssa:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Ruth Aurelia Butar:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Irma Septina Sitorus Pane:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Lili Melani:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization. **Noor Illi Mohamad Puad:** Writing – review & editing, Writing – original draft.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Muhammad Yusuf Abduh reports financial support was provided by Institut Teknolgi Bandung. Muhammad Yusuf Abduh reports a relationship with Bandung Institute of Technology that includes: employment and funding grants. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.focha.2025.100957.

Data availability

Data will be made available on request.

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